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An elevated pro-inflammatory cytokine profile in multiple chemical sensitivity

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Summary
Background: Multiple chemical sensitivity (MCS) is a medically unexplained condition characterized by reports of recurrent unspecific symptoms attributed to exposure to low levels of common volatile chemicals. The etiology of MCS is poorly understood, but dysregulation of the immune system has been proposed as part of the pathophysiology.
Objective: To compare plasma levels of cytokines in Danish MCS individuals with a healthy, sex- and age-matched control group.
Method: Blood samples were obtained from 150 un-exposed MCS individuals and from 148 age- and sex-matched healthy controls. Plasma concentrations of 14 cytokines, chemokines and growth and allergen-specific IgE were measured. All participants completed a questionnaire including questions on MCS, psychological distress, morbidities and medication use at the time of the study.
Results: Plasma levels of interleukin-1β, -2, -4, and -6 were significantly ($P < 0.001$) increased in the MCS group compared with controls, tumor necrosis factor-α was borderline significantly ($P = 0.05$) increased and interleukin-13 was significantly decreased ($P < 0.001$).
Conclusion: MCS individuals displayed a distinct systemic immune mediator profile with increased levels of pro-inflammatory cytokines and interleukin-2 and inverse regulation of Th2 associated cytokines interleukin-4 and interleukin-13 suggestive of low-grade systemic inflammation, along with a deviating Th2-associated cytokine response not involving IgE-mediated mechanisms.

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1. Introduction

Multiple chemical sensitivity (MCS) is a non-allergic chronic disorder characterized by reports of unspecific symptoms attributed to exposure to common volatile chemicals, such as fragranced consumer products, tobacco smoke, freshly printed papers or magazines (Graveling et al., 1999). Symptoms from the central nervous system (CNS) conveyed by headaches, dizziness, extreme fatigue, and concentration difficulties are common, symptoms from other organ systems, such as the airways and gastro-intestinal tract, are also frequently reported (Hausteiner et al., 2005; Lacour et al., 2005). Avoiding exposure to potential symptom-eliciting chemicals is a characteristic coping response in affected individuals, which includes avoiding public places and transportation, restricting social activities, and occupational considerations (Gibson et al., 2003). The prevalence of self-reported chemical sensitivity symptoms in population-based studies ranges from 9% to 33% (Hausteiner et al., 2005; Johansson et al., 2005; Berg et al., 2008), whereas physician-diagnosed MCS or reports of disabling consequences in the form of social and occupational disruptions are much lower, ranging from 0.5% to 6.3% (Berg et al., 2008; Kreutzer et al., 1999; Caress and Steinemann, 2003). There are currently no internationally accepted consensus criteria for MCS (Graveling et al., 1999; Das-Munshi et al., 2007); consequently, the diversity of applied case definitions in the scientific literature is high. Other labels have been ascribed to the condition, but MCS is widely used and will be used here without reference to any assumptions about the underlying etiology.

MCS is currently categorized as an unexplained disorder. The heterogeneity of the reported symptoms overlapping with other disorders, e.g. fibromyalgia (FMS) and chronic fatigue syndrome (CFS), has raised the question as to whether MCS constitutes a single disease entity with a specific etiology and pathogenesis. Several theories on the pathophysiology and risk factors involved in MCS have been suggested (Miller, 1997; Graveling et al., 1999; Yunus, 2008; Winder, 2002), but considering the clinical data available, no single theory alone has satisfactorily explained the presentations or multiple symptoms and no definitive conclusions can thus be drawn at this point (Das-Munshi et al., 2007; Graveling et al., 1999). However, evidence suggests that the symptoms are more likely to be caused by individual susceptibility factors than by an actual toxicological response (Bell et al., 2001; Das-Munshi et al., 2007) as MCS individuals do not show a typical dose—response relationship following exposure to symptom-triggering agents.

Dysregulation of the immune system has frequently been proposed as a pathophysiological mechanism likely to play a role in the etiology (De Luca et al., 2010; Meggs, 1993; Das-Munshi et al., 2007), and common MCS symptom-triggering compounds, such as formaldehyde, hydrocarbons and organochlorines, have been shown to suppress immune system functioning in humans (Vojdani et al., 1992). More supportive findings have been reported from unrelated studies, but the conclusions are inconsistent. To date, taken as a whole, immunological testing has failed to reveal any consistent pattern of reactivity or abnormalities indicative of common immunological deficiency in MCS (Kipen et al., 1992; Ziem and McTamney, 1997; Mitchell et al., 2000). One study by De Luca et al. (2010) reported increased levels of six immune-modulating cytokines in MCS individuals compared with healthy controls and abnormal serum levels of several biomarkers related to redox balance and metabolic functioning, which could suggest an impaired chemical defensive system and dysfunctional immune regulation (De Luca et al., 2010). Altered cytokine levels have also been studied in other medically unexplained disorders, such as FMS (Bazzichi et al., 2007; Kadetoff et al., 2012), CFS (Fletcher et al., 2009; Broderick et al., 2010; Maes et al., 2012) and gulf war syndrome (GWS) (Whistler et al., 2009; Skowera et al., 2004). Although results from these studies are inconclusive, several studies have reported findings of abnormal blood levels of pro-inflammatory cytokines in particular. Considering the complexities of MCS, it is unlikely that unaccompanied findings of abnormal serum levels of immunological mediators, such as cytokines alone, can be used to explain the pathophysiology of MCS. Nevertheless, they can provide useful information and serve as valuable pieces of the puzzle. Overall, the findings supporting an immunological component of MCS deserve further investigation.

The purpose of this study was to compare plasma levels of cytokines in un-exposed Danish MCS individuals with a healthy, sex- and age-matched control group. Target cytokines for the study were selected based on reported abnormalities found in similar studies and included several pro- and anti-inflammatory cytokines. Additional measurements of plasma allergen-specific immunoglobulin E (IgE) levels toward common inhalant allergens were included to account for possible differences in frequency of respiratory allergies between the two study groups.

2. Materials and methods

2.1. Study population

The study comprised 150 MCS individuals and 148 age- and sex-matched healthy controls aged 18—65 years. Participants with MCS were recruited among individuals who were registered in a research database at the Danish Research Centre for Chemical Sensitivities, Department of Dermato-allergology, Copenhagen University Hospital Gentofte, and through advertisements in patient organizations' newsletters and on the Danish Research Center's website. Age- and sex-matched healthy controls were recruited among staff members at Copenhagen University Hospital Gentofte, Denmark and staff members at Fredericia Hospital, Denmark and through flyers and posters at Copenhagen University Hospital Gentofte, Denmark as well as in the vicinity of the hospital.

2.1.1. Inclusion criteria for MCS individuals

All MCS individuals were screened for eligibility using the US Consensus Criteria for MCS and the revisions suggested by Lacour et al. (Lacour et al., 2005; 1999 Consensus on Multiple Chemical Sensitivity, 1999), which were operationalized as follows: (1) symptoms for at least 6 months; (2) symptoms occur in response to exposure to at least two of 11 common volatile chemicals; (3) presence of at least one symptom from the CNS and one symptom from another organ system; (4) symptoms causing significant lifestyle changes, (5) symptoms occur when exposed and lessen or resolve when the symptom-triggering agent is removed; (6) symptoms

Table 1 Observed plasma levels and analytical working range of the assay for each analyte included in the study. Results are expressed as pg/ml and data are presented as median values with lower and upper quartiles (P25/P75).

	MCS group (n = 150) Median (P25/P75)	Controls (n = 148) Median (P25/P75)	Assay Working Range LLOQ—ULOQ[a]
Interleukin-1β	43.1 (38.4/48/4)	37.4 (31.7/43.3)	0.2—556.0
Interleukin-2	247.3 (223.1/273.1)	230.6 (203.6/260.6)	4.5—4570
Interleukin-4	22.9 (16.6/24.8)	16.9 (14.3/18.8)	0.2—2611
Interleukin-5	301.0 (271.9/337.9)	306.15 (268.2/353.0)	5.0—4355
Interleukin-6	396.6 (350.3/457.8)	352.2 (288.0/404.6)	5.2—18,618
Interleukin-8/CXCL8[b]	12.7 (7.4/22.4)	12.5 (7.7/19.1)	1.9—26,403
Interleukin-10	220.8 (198.7/255.2)	222.3 (182.9/253.9)	1.1—11,850
Interleukin-12p70	40.98 (34.0/46.6)	40.1 (31.5/48.3)	1.7—3994
Interleukin-13	58.5 (53.0/66.2)	72.7 (60.9/81.3)	0.3—2406
TNF-α[c]	18.3 (15.1/21.7)	16.8 (14.0/20.6)	0.7—1814
Interferon-γ	108.9 (96.3/125.3)	107.6 (92.3/127.7)	0.2—2059
MCP-1/CCL2[d]	40.7 (31.7/50.1)	43.8 (35.8/52.6)	2.1—1820
PDGF-BB[e]	518.9 (244.4/1139.7)	402.5 (200.7/785.3)	7.0—51,933
VEGF[f]	49.4 (28.1/80.2)	42.4 (25.8/65.8)	5.5—56,237

[a] Lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ).
[b] Chemokine (C-X-C motif) ligand 8.
[c] Tumor necrosis factor-α.
[d] Monocyte chemotactic protein-1/chemokine (C-C motif) ligand 2.
[e] Platelet derived growth factor-BB.
[f] Vascular endothelial growth factor.

triggered by exposure levels that do not induce symptoms in other individuals who are exposed to the same levels.

2.1.2. Inclusion criteria for controls

Did not fulfill any of the study criteria for MCS or have any close family member, i.e. parent, grandparent, sibling or child with MCS and did not share housing with an MCS affected individual.

2.1.3. General exclusion criteria

Exclusion criteria were pregnancy or nursing at the time of the study, and physician diagnosis of FMS or CFS.

2.2. Blood sampling

Venous blood samples of 4 ml were collected in anticoagulant disodium EDTA tubes (Greiner Bio-One, Kremsmünster, Austria). To minimize degradation, samples were immediately spiked with a protease inhibitor cocktail containing 400 μg 4-(2-aminoethyl)benzenesulfonyl fluoride, hydrochloride (Pefabloc Sc) (Roche, Basel, Switzerland), 200 KIU aprotinin (Sigma—Aldrich, St. Louis, MO, USA) and 28 nM dipeptidyl peptidase-IV inhibitor (Sigma—Aldrich). Plasma was recovered by centrifugation at 2500 × g for 10 min at 4 °C and stored at −80 °C until batch analysis.

2.3. Fluorescence-based multiplexed cytokine assay

All immunoassays were performed using fluorescently labeled microsphere beads with suspension array system Bio-Plex® kits (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and analyzed on a BioPlex 200 (Bio-Rad). The cytokines interleukin-1β (IL-1β), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-12p70 (IL-12p70), interleukin-13 (IL-13), tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) were quantified simultaneously as a 10-plex using the Bio-Plex® Human Cytokine Assays (Cat# 171-A1001P), while interleukin-8/chemokine (C-X-C motif) ligand 8 (IL-8/CXCL8), monocyte chemotactic protein-1/chemokine (C-C motif) ligand 2 (MCP-1/CCL2), platelet derived growth factor-BB (PDGF-BB) and vascular endothelial growth factor (VEGF) as a 4-plex assays (Cat# L50006YX76). For each analyte, the lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) according to the manufacturer are shown in Table 1.

2.4. Immunoglobulin analyses

Plasma samples were analyzed for specific IgE antibodies to a mix of common inhalant allergens using the Phadiatop test for allergic sensitization (to birch, timothy, mugwort, cat, dog, horse, molds [*Cladosporium herbarum*], and house dust mite [*Dermatophagoides pteronyssinus*]) ImmunoCAP 250 (Thermo Fisher, Uppsala, Sweden (formerly Phadia/Pharmacia)), reported in kUA/L and calibrated to the WHO 75/502 IgE standard. Values equal to or greater than 0.35 kUA/L were considered indicative of sensitization and used as threshold for a positive or negative response in this study.

All tests were performed according to the manufacturers' specifications and the laboratory standards according to ISO-15189 certification. All laboratory tests were performed by personal independent of the study and test results were only made available to the investigators when all samples had been analyzed.

2.5. Questionnaires

Background characteristics in terms of sex, age, morbidities and medication use were collected from all study participants. The following questionnaires were included.

2.5.1. The Quick Environmental Exposure and Sensitivity Inventory

The Quick Environmental Exposure and Sensitivity Inventory (QEESI) was developed as a screening instrument for MCS designed to facilitate history taking. It consists of four scales, each containing 10 items and a score ranging from zero to 100 including symptom severity, chemical intolerances, other intolerances, and life impact. In addition there is a fifth scale, a masking index (scores ranging from zero to ten) that offers some assessment on ongoing exposures that may affect individual awareness of intolerances and the intensity of responses to environmental exposures (Miller and Prihoda, 1999b). A Danish translation of the QEESI has been evaluated in relation to internal consistency, test-retest reliability, and to describe sensitivity and specificity and establish normative data (Skovbjerg et al., 2012; Miller and Prihoda, 1999b). Other studies have also reported satisfactory psychometric properties of the QEESI (Skovbjerg et al., 2012; Miller and Prihoda, 1999a). Only the symptom severity scale, chemical intolerances and life impact scales were included in the study.

2.5.2. Symptom Checklist 92

Symptom Checklist 92 (SCL-92) subscales for depression and anxiety were included. These subscales consist of 35 items where responses are rated on a 5-point Likert scale ranging from *not at all* to *very much*. A Danish translation of the SCL-92 has been validated in a Danish general population and normative data have been established (Olsen et al., 2004).

2.5.3. Socioeconomic position

Socioeconomic position was measured as occupational social class, which includes standardized questions on school education, vocational training and occupation. Data on socioeconomic position were categorized into a descending scale with occupational social class **I**—**V**. Occupational social class **I** includes professionals and executives; **II** medium-level white-collar employees; **III** low-level white-collar employees; **IV** skilled workers; and **V** semi-skilled and unskilled workers. An additional three groups were formed including: **VI**, individuals receiving pension or disability benefits; **VII**, students; and **VIII**, "outside classification" if the data did not provide enough information about education and current occupation. This approach is in accordance with the standard of the Danish National Institute of Social Research, which is similar to the British Registrar General's Classification **I**—**V** (Christensen et al., 2006).

2.5.4. Asthma

Questions on asthma were adapted from the first stage questionnaire of the European Community Respiratory Health Study (ECRHS) (Pekkanen et al., 2005). Asthma was defined according to criteria employed by the ECRHS as an affirmative answer to at least one of the following questions: (1) Have you been woken by an attack of shortness of breath at any time in the last 12 months? (2) Have you had an attack of asthma in the last 12 months? (3) Are you currently taking any medicine (including inhalers, aerosols or tablets) for asthma? (Sunyer et al., 2004).

2.6. Approval

All research reported here was approved by the Research Ethics Committee of Copenhagen County (Protocol-number H-3-2011-029) and by the Danish Data Protection Agency (ID-number GEH-2011-018). Signed informed consent was obtained from all study participants.

2.7. Statistical analyses

Descriptive statistics for the two groups were generated. Due to skewed distributions medians are presented for QEESI and SCL-92. Statistical analyses were conducted using SAS version 9.3; the level of significance was set at 0.05.

Multiple linear regression analyses using the SAS GLM procedure were conducted with the 14 plasma levels of cytokines, chemokines and growth factors as the dependent variables and the grouping variable, i.e. MCS or control, as the explanatory variable. Due to skewed distributions, the dependent variables were log-transformed prior to statistical analyses. All analyses were adjusted for sex, age, Body Mass Index (BMI), asthma, specific IgE (Phadiatop), smoking, and dichotomized SCL-92 depression and anxiety scores. To test whether the assumptions for linear regression analyses were met, model control was performed examining the residuals for normal distribution and homogeneity of variance.

3. Results

3.1. Characteristics of study groups

Characteristics of the two groups are shown in Table 2. More women than men participated in the study. A small but statistically significant difference was seen in age between the two groups with a mean of 48.1 years in the control group versus 52.6 years in the MCS group. The median QEESI scores revealed a significantly higher score on all three scales, i.e. symptom severity, chemical intolerances and life impact, for the MCS group compared with the control group. The MCS group reported more depressive and anxiety symptoms as well as poorer overall self-rated health. The socioeconomic data revealed significant differences between the two group's occupational circumstances with 50.7% of the MCS groups receiving a pension or disability benefits compared with 3.4% in the control group. Plasma levels of specific IgE antibodies revealed no significant differences between the two groups (Table 2).

The distribution of morbidities other than MCS is presented in Fig. 1 for each group. Compared with the control group, the MCS group reported an overall higher comorbidity level and a higher consumption of prescribed medication. These results are in line with the MCS group's self-rated

Table 2 Observed characteristics of the MCS and the control groups.

	MCS group (n = 150)	Control group (n = 148)	
Sex male/female (n/%)	20(13.3)/130(86.7)	33(22.3)/115(77.7)	P-value[a]
Age (mean/sd[b])	52.6 (9.1)	48.1 (9.9)	<0.001
Weight (mean/sd)	70.6 (17.1)	71.7 (14.1)	0.55
QEESI (median)			P-value[c]
Symptoms	49.0	6.0	<0.001
Chemical intolerances	75.5	3.0	<0.001
Life Impact	64	0.0	<0.001
SCL-92 (median)			P-value[c]
Depression	0.54	0.12	<0.001
Anxiety	0.50	0.10	<0.001
Self-rated health (n/%)			P-value[d]
Excellent/very good	24 (16.0)	114 (77.0)	<0.001
Fair	60 (40.0)	24 (16.2)	
Not too good/bad	57 (38.0)	4 (2.7)	
Missing	9 (6.0)	6 (4.1)	
Employed (n/%)	68 (45.3)	143 (96.6)	
Occupational social class (n/%)			P-value[d]
I + II	15 (10)	35 (23.6)	<0.001
III + IV	46 (30.7)	98 (66.2)	
V + VI	6 (4.0)	7 (4.7)	
VII	76 (50.7)	5 (3.4)	
Missing	7 (4.7)	3 (2.0)	

QEESI: Quick Environmental Exposure and Sensitivity Inventory; **SCL-92**: Symptom Checklist-92.
Occupational social class: I + II, professionals and executives and medium-level white-collar employees; **III + IV**, low-level white-collar employees and skilled workers; **V + VI**, semi-skilled and unskilled workers and students; **VII**, receiving disability benefits.
[a] Independent samples t-test for equality of means (total) between MCS and control groups.
[b] Standard deviation.
[c] Mann—Whitney test (total) comparing MCS and control groups.
[d] Chi-square test comparing MCS and control groups.

health score being significantly lower, thus suggesting an overall poorer health status compared with the control group (Table 2). Additionally, approximately 40% of the control group reported no morbidities, while for the MCS group only around 9% reported having no morbidities other than MCS.

3.2. Cytokine patterns in MCS individuals

Plasma levels of all 14 cytokines, chemokines and growth factors included in the study are presented in Table 1 and the results of the subsequent multiple linear regression analyses

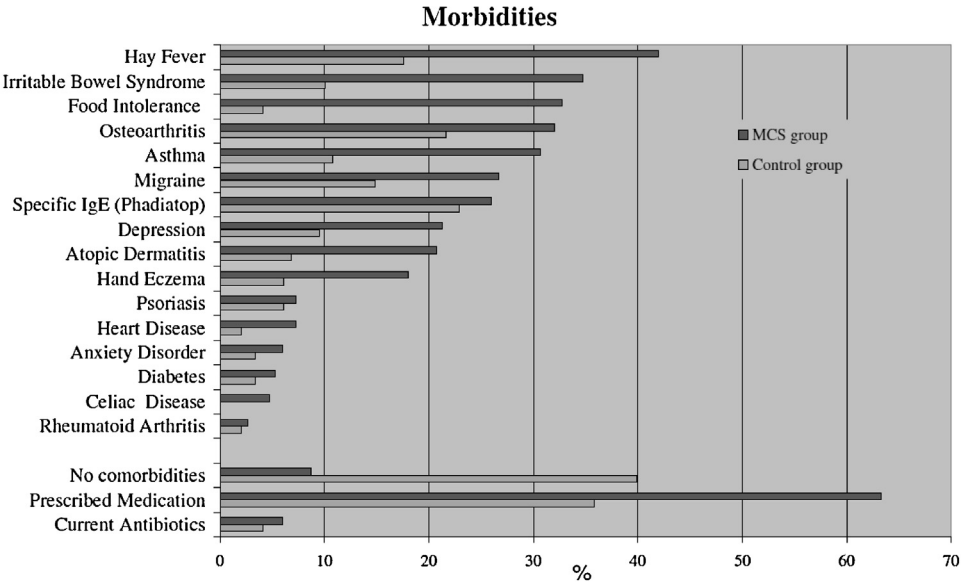

Figure 1 Morbidities reported in the two study groups including proportion of positive Phadiatop tests, use of prescribed medications and current use of antibiotics. Data are expressed as % of individuals affected by each single category within each study group.

Table 3 Results of multiple linear regression analyses with interleukin-1β, -2, -4, -6, -13 and tumor necrosis factor-α as the dependent variables and group, sex, age, Body Mass Index (BMI), asthma, specific IgE, smoking, and dichotomized SCL-92[a] depression and anxiety scores as the explanatory variables.

BETA (SE[b])	PR > F^c	
Interleukin-1β		
Group[d]	0.15 (0.03)	<0.0001
Sex	0.08 (0.04)	0.06
Interleukin-2		
Group	0.07 (0.03)	<0.009
Sex	0.06 (0.03)	0.11
Interleukin-4		
Group	0.29 (0.03)	<0.0001
Sex	0.07 (0.04)	0.08
Interleukin-6		
Group	0.17(0.04)	<0.0001
Sex	0.11(0.05)	0.02
Interleukin-13		
Group	−0.02(0.03)	<0.0007
Gender	0.09(0.04)	0.01
Tumor necrosis factor-α		
Group	0.08	0.05
Sex	0.14	0.01

[a] Symptom Checklist-92.
[b] Standard error.
[c] Level of significance is 0.05.
[d] MCS group versus control group.

are summarized in Table 3. There was a statistically significant linear relationship with group with increased levels of IL-1β, IL-2, IL-4, IL-6 and decreased levels of IL-13 in MCS individuals compared with controls (Fig. 2). For TNF-α, we found the same trends as for IL-1β, IL-2, IL-4, IL-6, but only with borderline significance (Fig. 2). We found no differences in plasma levels for IL-5, IL-8/CXCL8, IL-12p70, IL-10, IFN-γ, MCP-1/CCL2, PDGF-BB and VEGF. Sex also influenced cytokine levels independent of MCS, with women showing higher IL-6, IL-13 and TNF-α levels compared with men (Table 3). Notably, the two Th2-associated cytokines IL-4 and IL-13, were found inversely regulated in the MCS group although these two cytokines have several mediator functions in common. In both groups, IL-13 was produced at higher molecular levels than IL-4 (Table 1), but the ratio of IL-4 to IL-13 was significantly ($P < 0.0001$) higher in the MCS group, with a mean IL-4/IL-13 ratio of 0.378 ± 0.0366 versus 0.233 ± 0.0337 in the control group.

3.3. Associations between anxiety or depression and cytokine levels in MCS

Additionally, subgroup analyses were conducted to investigate a possible association between anxiety or depression and altered cytokine levels in MCS individuals. Due to the low number of men participating in the study, only female MCS individuals were included in the analysis. Levels of IL-1β, IL-2, IL-4, IL-6, IL-13 and TNF-α were compared between female MCS individuals with an SCL-92 score above cut-off for anxiety ($n = 25$) or for depression ($n = 21$) and individuals with an SCL-92 score below cut-off on these scales. The analyses showed that female MCS individuals with a score above cut-off for depression had statistically significantly higher levels of IL-2 ($P = 0.042$). No other significant results were seen (data not shown).

4. Discussion

The objective of this study was to examine plasma levels of selected cytokines in Danish MCS individuals in comparison with a healthy, sex- and age-matched control group. More women than men participated in the study, which is comparable to earlier reports of MCS being more prevalent among women (Das-Munshi et al., 2007). The MCS group scored significantly higher on the three QEESI scales compared with the control group, suggesting that the case criteria for inclusion in the study were successful. There was a small, but significant difference in age between the two study groups. However, as revealed by the multiple linear regression analyses, group differences in cytokine levels were independent of age. Age is therefore unlikely to influence the results. The MCS group also reported more depressive and anxiety symptoms as well as poorer general self-rated health. This is consistent with the increased levels of non-MCS morbidities reported by MCS individuals, demonstrating the disabling and complex nature of this multi-symptomatic disorder. The frequency of positive phadiatop tests was comparable between the two populations, which is in line with earlier findings (Kipen et al., 1992; Berg et al., 2011) and strongly supports that the symptoms experienced by MCS individuals after inhalation of common volatile chemicals are not mediated by enhanced IgE. Additionally, since the proportion of participants on antibiotics at the time of inclusion and the number of positive phadiatop tests (against specific IgE) are comparable between the two study groups, these factors are believed not to have any influence on the levels of plasma cytokines.

This study supports earlier findings of abnormal levels of several immune-modulating cytokines in un-stimulated MCS individuals (De Luca et al., 2010). Of the 14 analytes included in this study, plasma levels of IL-1β, IL-2, IL-4 and IL-6 were found to be statistically significantly increased in MCS, TNFα was borderline enhanced, whereas IL-13 was downregulated. Due to the inverse relationship in levels of the Th2-associated cytokines IL-4 and IL-13 in the MCS group, the ratio of IL-4/IL-13 was found to differ significantly in MCS versus controls. For the remaining analytes, plasma levels were comparable. Our data do not replicate the findings of De Luca et al. (2010) that demonstrated altered levels of IL-8/CXCL8, IL-10, IFN-γ, MCP-1/CCL2, PDGF-β and VEGF. However, the inconsistencies between the two studies could be explained by methodological differences. The two studies used the same analytical method from the same producer, but some differences are notable. In the study by De Luca et al. (2010) a standard Human Cytokine 27-plex Assay was used rather than the high sensitivity 10-plex and 4-plex assay used in the present study. When analyzing many substances simultaneously, it is often necessary to compromise with dilution of samples and detection/quantification limits. This has been discussed in recent studies that revealed critical

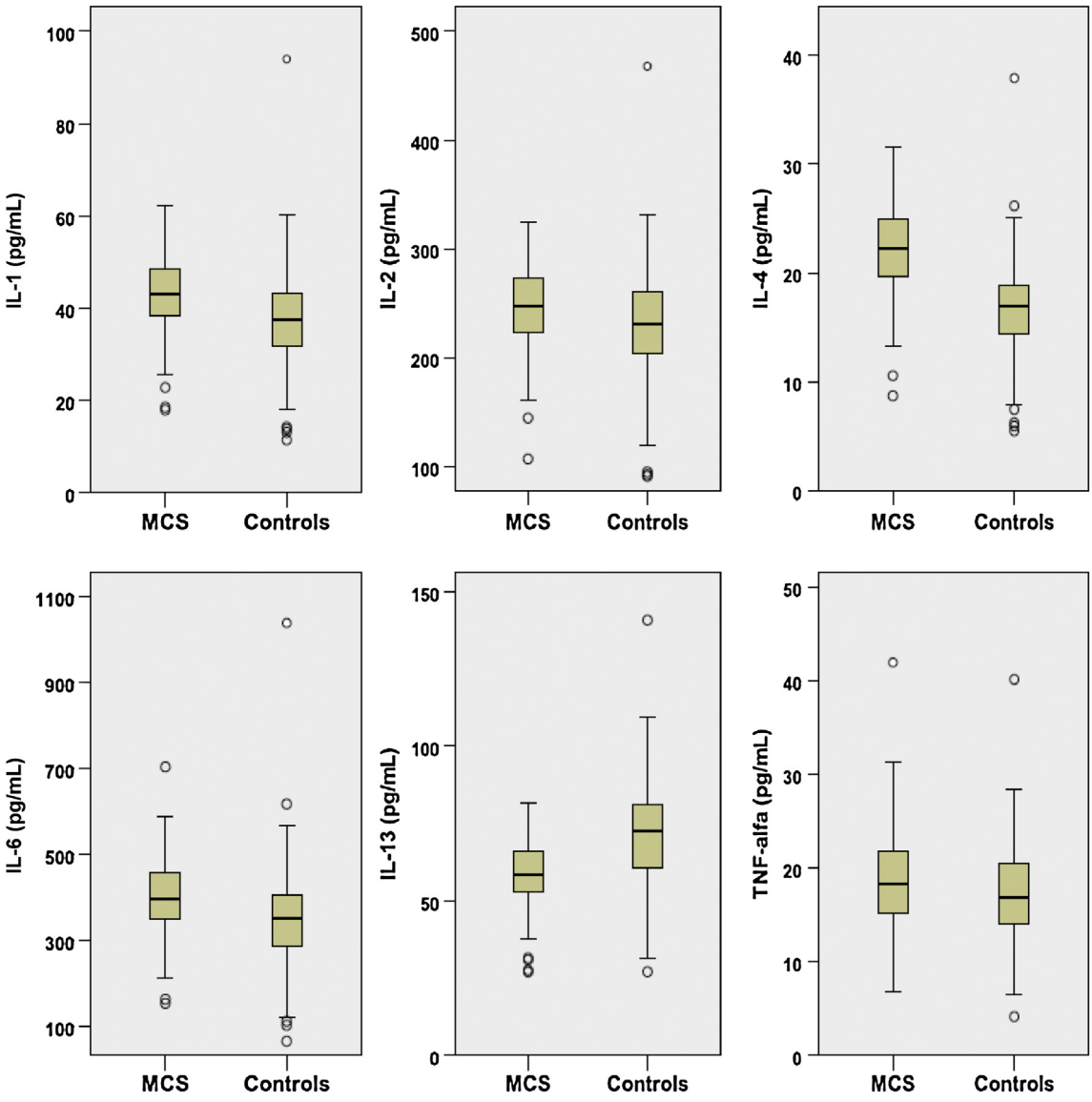

Figure 2 Plasma levels of the six analytes identified as being either significantly increased (interleukin-1β (IL-1β), IL-2, IL-4, IL-6 and tumor necrosis factor-α (TNF-α)) or significantly decreased (IL-13) in the MCS group ($n = 150$) compared with the control group ($n = 148$). Values are presented as median, lower and upper quartiles (upper and lower limit of the box) and whiskers represent the highest and lowest values that are not outliers. Outliers are defined as more than 1.5 times the inter-quartile range and are presented by individual circles.

detectability and/or reproducibility limitations of the 27-plex broad range assay for a number of cytokines including IL-1β, IL-2, IL-4, IL-5, IL-10, IL-13 and TNFα (Chaturvedi et al., 2011; Biancotto et al., 2012). To overcome these possible analytical limitations, we used a more sensitive assay for the cytokines found in low concentrations and split the test in two portions. Furthermore, De Luca et al. (2010) did not protect samples from naturally occurring proteolysis by addition of a protective mixture of protease inhibitors. Many cytokines are surprisingly stable but not all, and this error factor is otherwise almost impossible to account for as individual analytes will be degraded at different velocities. It is likely that these preventive measures can explain at least some of the conflicting findings. The case definition in De Luca et al. (2010) is presented in little detail and the statistical analyses are unadjusted for possible confounders, which hinders direct comparison between the two studies (O'Connor and Irwin, 2010; Chaturvedi et al., 2011). Nevertheless, both studies report altered levels of circulating cytokines in MCS. The cytokine differences in MCS seen in this study are in line with findings from other medically unexplained disorders often associated with MCS, such as FMS, CFS and GWS. Altered cytokine levels have been studied more intensively in these disorders, and although conclusions have revealed a high degree of inconsistency, several studies have reported differences in cytokine levels. In particular, increased pro-inflammatory and Th1/Th2-related cytokines, such as IL-1α, IL-1β, IL-2, IL-4 and IL-5, IL-6, TNF-α and IFN-γ, have been reported in individuals with FMS, CFS and GWS (Bazzichi et al., 2007; Kadetoff et al.,

2012; Fletcher et al., 2009; Broderick et al., 2010; Maes et al., 2012; Whistler et al., 2009; Skowera et al., 2004). Likewise, in two separate studies of CSF patients, IL-13 and IL-15 levels were found to be reduced (Fletcher et al., 2009; Broderick et al., 2010), concordant with our finding for IL-13 in MCS. Abnormal levels of IL-8/CXCL8/and the anti-inflammatory cytokine IL-10 have also been reported in a number of studies, but findings have been contradictory (Fletcher et al., 2009; White et al., 2010; Kadetoff et al., 2012).

4.1. Cytokine patterns in MCS

The variations in cytokine levels identified in the MCS group could be indicative of elevated immune activation or a chronic inflammatory stage mediated by increased levels of the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α, all recognized as cardinal biomarkers for chronic low-grade inflammation when enhanced without concurrent infection (Feghali and Wright, 1997). This is further supported by the increased IL-2 levels. IL-2 is involved in proliferation and differentiation of lymphocytes, playing a key role in expansion of T-cell, NK-cell, and B-cell populations, including regulatory T cells, during various phases of an immune response (Boyman and Sprent, 2012). IL-2 is therefore considered a good indicator of an activated immune system. The Th1-promoting cytokine IL-12p70 and the neutrophil attractant IL-8/CXCL8 were also measured in this study but did not differ between groups. IL-12p70 and IL-8/CXCL8 are often increased together with IL-1β, IL-6 and TNF-α as mediators of the acute-phase response to an infection. However, in contrast to IL-1β, IL-6 and TNF-α, which mediate both local and systemic effects, the effector functions of IL-8/CXCL8 and IL-12p70 are more isolated to specific tissue areas affected by inflammation (Parham, 2009). Increased levels of IL-8/CXCL8 and IL-12p70 are therefore less likely to be measured in blood. This is a general point to take into consideration when interpreting our systemic cytokine data because the cytokine pattern found in peripheral blood reveals only parts of the cytokine signature that are in play at the affected site of inflammation.

The Th2-associated cytokines were found to be diversely expressed in MCS individuals, with IL-4 being significantly increased, IL-13 significantly reduced, and IL-5 levels similar to those found in the control group. Overproduction of IL-4 has classically been associated with allergies and several of the co-morbidities frequently reported by MCS individuals, such as asthma, hay fever and atopic dermatitis are Th2-related (Parham, 2009). However, measures of aeroallergen-specific IgE levels revealed no differences between the two groups, speaking against a critical effect of the increased plasma IL-4 levels on IgE-isotype switching in MCS. In addition, subsequent regression analysis showed no association between the individuals reporting Th2-related disorders (asthma, hay fever, atopic dermatitis) and increased IL-4 levels. This is further supported by the significantly enhanced ratio of IL-4 to IL-13 found in the MCS group, which speaks against a common Th2-driven immune response occurring since IL-13 has several Th2 associated mediator functions similar to those of IL-4. It is therefore possible that the increased plasma IL-4 levels in MCS individuals could be derived from tissue-located IL-4 producing cells, such as eosinophils or mast cells, which may infiltrate exposed tissue types, e.g. lungs or nasal lamina propria, during a chemical exposure. Although speculative, the increased IL-4 levels might then be an indicator of increased tissue remodeling taking place to enhance tissue repair in the exposed tissue areas. Accordingly, the finding of decreased IL-13 levels in MCS suggests variable effects of this otherwise Th2-related cytokine and IL-13 is also known to mediate physiologic changes in many tissue types and to stimulate mucus production in, e.g. airway epithelium cells (Locksley, 2010). It has been reported that IL-13 changes the mucosal epithelium from being an absorptive barrier to a mucus secreting one (Danahay et al., 2002), thus tipping the balance toward increased absorption at low IL-13 levels. IL-13 is also involved in suppression of pro-inflammatory cytokine production from macrophages within inflamed tissue (Minty et al., 1993) as well as in inhibition of Th1 propagation in lymph nodes and promotion of B-cell activation (Punnonen et al., 1993). The decreased systemic levels of IL-13 may therefore be an indicator of diminished barrier protection, if the reduced IL-13 levels implicate reduced mucus production at mucosal surfaces, and also of reduced inhibition of pro-inflammatory cytokine production within tissues.

4.2. Sickness behavior and mood disorders

Many of the symptoms reported in MCS resemble inflammation-mediated sickness behavior, consisting of an alert system encompassing a highly organized whole-body strategy to fight the numerous types of infections humans are exposed to (Hart, 1988). The typical symptoms include malaise, anorexia, joint and muscle aches, general fatigue, cognitive impairment, activation of the hypothalamic-pituitary-adrenal axis as well as reduced appetite and withdrawal from physical activities and social interactions (Hart, 1988; Dantzer, 2009). Sickness behavior is triggered by release of pro-inflammatory cytokines produced by activated innate immune cells, most importantly IL-1α, and the three cytokines IL-1β, IL-6 and TNF-α, which was found increased in MCS individuals in the present study. A sudden elevation of these cytokines is known to have a profound effect on cellular function throughout the body, affecting both behavior and mood via cytokine-induced changes in CNS functioning (Hart, 1988; Dantzer et al., 2008). Although cytokine-promoted sickness behavior is a natural and temporarily occurring phenomenon (Dantzer, 2009; Dantzer et al., 2008), it can become pathologic when it occurs out of context, i.e. in the absence of an inflammatory stimulus or when it is exaggerated in intensity or duration, e.g. being chronic in nature (Dantzer, 2009).

There is also growing evidence pointing to an association between sustained long-term production of circulating pro-inflammatory cytokines and symptoms characteristic of depression and anxiety (Dantzer et al., 2008), which are often associated with MCS (Bornschein et al., 2002; Caccappolo-van et al., 2002; Hausteiner et al., 2003). This association is also confirmed in this study with the MCS group reporting more depressive and anxiety symptoms (Table 2 and Fig. 1). Although speculative, it is plausible that the increased frequency of mood disorders experienced by MCS individuals could be affected or exaggerated by the altered cytokine profile identified.

It is not possible to draw any conclusions based on the present findings regarding a possible association between sickness behavior or mood disorders and MCS symptoms and the observed differences in cytokine levels. But considering the role of pro-inflammatory cytokines in both inflammation-related sickness behavior and mood disorders (Dantzer et al., 2008; Capuron et al., 2003, 2004) it is a possible avenue for future investigations, since both De Luca et al. (2010) and the present study have identified increased levels of pro-inflammatory cytokines in individuals with MCS, suggestive of a chronic low-grade inflammation.

Altogether our results provide confirmative evidence for an altered cytokine profile in MCS. The inclusion of additional immunological mediators may have strengthened the study by providing a more comprehensive characterization of the cytokine profile. However, this was an exploratory study and our results suggest that a more detailed mapping of the cytokine profile is a question for future studies. As no objective measures exist for establishing the presence and degree of MCS, the inclusion procedure as well as background characterization necessarily had to rely on self-reports. How to define MCS is a continuously and challenging question in clinical end epidemiological studies in this field, and may give rise to uncertainties regarding whether MCS in fact constitutes a clinical entity and how to compare results across studies. We approached this question by using a screening interview for inclusion and the QEESI, which has demonstrated as a reliable instrument for MCS (Skovbjerg et al., 2012; Miller and Prihoda, 1999a) and based on the age and sex distribution of the cases and the QEESI scores in the MCS group (Table 2), we believe that our results are generalizable.

5. Conclusion

Collectively, our study reports a different immunological profile in MCS individuals pointing toward an increased secretion of pro-inflammatory cytokines and an enhanced IL-4/IL-13 ratio. To date, several positive findings have been reported, but conclusions have remained inconsistent and, on the whole, immunological testing has failed to reveal any consistent pattern of immunological reactivity or immunological abnormalities indicative of a specific immunological deficit in MCS. The strengths of our study are the large sample size, the careful case definition and the analytical procedures, which taken together support our findings of statistically significant differences in cytokine levels in MCS compared with a relevant control group after adjusting for possible confounding factors. The clinical relevance of these findings can be only speculative at this point and cannot currently be utilized in the diagnosis of MCS, but the possible role of these immunological differences in MCS needs further investigation

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Conflicts of interest

None declared.

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